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Nora E. Dalbey.

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A Comparative Anatomical Study of Some Species of *Xanthium.*

BY NORA E. DALBEY.

Plates XVI to XXII.

INTRODUCTION.

EIGHT species of *Xanthium* are now recognized in the territory east of the one hundredth meridian. One species, which in the first edition of the Illustrated Flora of the United States and Canada, by Britton and Brown, was called *Xanthium canadense,* is now known to be two distinct species, *Xanthium americanum* Walter, and *Xanthium pennsylvanicum* Wallroth

In 1912 Prof. Charles A. Shull, of the University of Kansas, for the purpose of securing pure lines for experimental work, made a collection of the seeds of the three main types of *Xanthium* found in the fields about Lawrence. Of the three types, one resembled closely *Xanthium pennsylvanicum,* another agreed well with the description of *Xanthium americanum,* and the third was a form distinguished by short globose burs, to which he gave the provisional name of *Xanthium globosum.* He thinks this type is new, as it has never been described among the published species of *Xanthium.* Professor Shull selected seeds from one plant of each type, and planted them in pots in the laboratory, and about the first of June transferred them to the breeding grounds. It was expected that the plants would show the characteristics of hybrids, but instead, they developed with uniformity, showing three distinct types,

and it was from these plants, in the early part of September, 1913, that the material for this study was secured.

LEAF.

There are very distinct external differences between the leaf of *X. globosum* and of *X. pennsylvanicum.*

The leaves of *X. globosum* are dark green and have a much crinkled surface, while those of *X. pennsylvanicum* are lighter in color and have a comparatively even surface. The leaf of *X. americanum* is in its superficial aspect very much like that of *X. globosum.* There are no very striking differences in leaf structure shown in either cross or tangential sections (figs. 1, 2, 3, 4, 9), but some minor differences occur which are constant.

The leaf of each species is petiolate, with about ten to fourteen vascular bundles entering from the stem. The leaf is entered by three large veins, each containing about the same number of vascular bundles (fig. 12). In the apex of the leaf, and in the apex of each deeply cut tooth on the margin, the veins end in clusters of tracheids (fig. 10, *W*). The midrib and other large veins of the leaf contain a very large amount of thin-walled mesophyll cells, scanty in cell contents (figs. 11, *B;* and 12, *J*). The vascular bundles contain a relatively large amount of phlëm (fig. 21, *X*). The epidermis of the midrib is uniform in shape and size (fig. 15, *N*), with a heavier cuticle layer than on other parts of the leaf (fig. 11). Beneath the epidermis of the midrib there is a layer of collenchyma cells from three to six cells in thickness (fig. 11, *C*).

Rather large border parenchyma cells, which are almost empty of cell contents, surround the vascular bundles of the veins (figs. 1, *A;* 2, *D;* 3, *G*), and extend in plates to the margin above and below (fig. 4, *L*). Resin ducts, in cross section, surrounded by, on the average, six secreting cells, occur in the parenchyma in the midrib and surrounding the larger veins (fig. 12, *G*).

The leaf of each species has the dorsi-ventral differentiation shown in figures 4 and 9. In *X. pennsylvanicum* the palisade tissue has a length in cross section of 3 cells, on the average (fig. 9), while the average in *X. globosum* is 2 cells, and in *X. americanum* 2.5 cells (fig. 4).

The ratio of the area, in cross section, of the palisade tissue to the area of spongy parenchyma is in *X. pennsylvanicum*

1 to 1, and in *X. globosum* and *X. americanum* 1 to 1.6. In *X. pennsylvanicum* the palisade cells of one section varied in length from .033 mm. to .062 mm. (figs. 5 and 6). In the other species this variation was not so great, being .030 mm. to .037 mm. in *X. globosum* (fig. 7), and .030 mm. to .045 mm. in *X. americanum* (fig. 8).

In cross sections of the leaves of the different species, which had been treated with alcannin, the mesophyll cells contained large bodies which stained a bright red. When the sections were put into ether and then treated with alcannin the same bright-red bodies were seen, but when treated to boiling in Fehling's solution, and left in the solution several hours at a temperature of about 35° C., copper oxide crystals replaced these round bodies. Since the reaction for sugar did not occur except after long contact with Fehling's solution, the bodies are probably glucosides, which yield sugar after hydrolysis by long standing in Fehling's solution.

The cells of the epidermis are rather irregular in size (fig. 4, *J*). In cross section the average diameter parallel with the circumference is in *X. pennsylvanicum* .015 mm., in *X. americanum* .0131 mm., and in *X. globosum* .0135 mm. In tangential section the epidermal cells of *X. pennsylvanicum* are more irregular in shape than the epidermal cells in the other species (figs. 16, 17, 19).

A ridged cuticle layer covers the outer cell walls in each species.

Stomata of the usual type (figs. 14, *M;* 16, *O*) occur on both sides of the leaf, more numerous in *X americanum* than in the other species, as the following tabulation will show:

	Upper side.	Under side.
X. pennsylvanicum	243.5 to sq. mm.	205.8 to sq. mm.
X. americanum	309.9 to sq. mm.	270.5 to sq. mm.
X. globosum	196.0 to sq. mm.	166.0 to sq. mm.

In cross section the guard cells are much smaller than the adjacent epidermal cells, as the following comparison shows:

	Guard cells.	Adjacent epidermal cells.
X. pennsylvanicum	.005 mm. diam.	.017 mm. diam.
X. americanum	.007 mm. diam.	.030 mm. diam.
X. globosum	.005 mm. diam.	.020 mm. diam.

Numerous trichomes of two different forms occur on both surfaces of the leaf (fig. 22, *E* and *F*).

The most conspicuous one is a large hair with an average length of .14 mm., composed of a basal cell, one or sometimes

two middle cells, and a long, pointed terminal cell (fig. 24), with the outer wall of all the cells sometimes showing distinct papillæ. Below the basal cell there are a number of very regular cells (figs. 24, *I;* 17, *Q*), larger than the other cells of the epidermis, which in tangential section appear to form a rosette (fig. 17, *Q*). Both forms of hairs are essentially alike in the three species. The number of the large hairs varies in the three species: in *X. pennsylvanicum* 22.4 per sq. mm., in *X. americanum* 53.8, and in *X. globosum* 46.7. The small linear hairs are numerous over the veins. They are composed of from two to eight cells of about equal size (fig. 22, *E*).

The hairs are distributed in definite relation to the veins, for both large and small hairs always occur in connection with the water-storage tissue above and below the veins (fig. 23, *G* and *H*), those above the intersection of two veins being usually larger (fig 23). The cuticle extends unbroken over all the hairs.

STEM.

The young stems are slightly five-angled (figs. 25, 26 and 27), a character which seems to be a little more evident in *X. pennsylvanicum* (fig. 26) than in *X. americanum* and *X. globosum,* while older stems are nearly circular in cross section. At the angles, in the young stems, the parenchyma of the pericycle is not so wide in cross section as elsewhere (figs. 25 to 27). At these angles the vascular bundles are larger (figs. 27 and 28), with xylem in which the rows of vessels show marked radiation (fig. 28). Stems from the three species, of about the same size and age, in cross section show a difference in the comparative amount of each tissue, as indicated in the following tabulation giving radial diameters:

	Collenchyma.	Parenchyma of pericycle.	Phloëm.	Xylem.	Pith.
	mm.	*mm.*	*mm.*	*mm.*	*mm.*
X. pennsylvanicum	.125	.365	.084	.490	4.531
X. americanum	.125	.318	.131	.693	3.876
X. globosum	.156	.375	.156	1.093	3.843

The pith of each species is composed of rather thin-walled, pitted cells (figs. 29, 30 and 31). There is a very slight variation in both longitudinal and transverse diameters of the pith cells of the three species:

	Longitudinal diameter.	Transverse diameter.
X. pennsylvanicum	.110	.070
X. americanum	.132	.071
X. globosum	.118	.076

The cell walls vary in thickness from .0015 mm. to .002 mm., and are cellulose, except those near the wood, which have lignified walls. In each species the average diameter of the pith in number of cells varies from 30 to 35.

Clusters of calcium oxalate crystals, with an average diameter of .015 mm., are regularly distributed throughout the pith (figs. 32, *N*; 33, *O*). The average number per sq. mm. seen in cross section varies in the three species: in *X. pennsylvanicum* 1000, in *X. americanum* 234.5, and in *X. globosum* 493.8.

The pith cells of each species contain a large amount of stored-up material in the form of glucosides. Sections boiled in Fehling's solution did not show a reaction for sugar, but when sealed on slides in Fehling's solution, and kept at a rather high temperature for about forty-five minutes, crystals of copper oxide appeared, indicating the hydrolysis of glucosides.

The wood increases in size by the interpolation of new bundles, the bundles of one section of a growing stem showing a large variation in size (fig. 27). In *X. americanum* and *X. globosum* the ray cells between the bases of the bundles are elongated parallel to the circumference (fig. 41, *N*). *X. pennsylvanicum* does not show this characteristic.

In cross section the vessels are seen in radial rows (figs. 38, 39 and 40), with the rows of vessels in the large bundles at the angles of the stem (fig. 28) separated by a greater amount of wood parenchyma than in other bundles of the same stem (fig. 27). The vessels varied in diameter in a single bundle from .010 to .060 in *X. pennsylvanicum*, .017 to .052 in *X. americanum*, and .015 to .038 in *X. globosum*. The species vary in the per cent of stem area made up of vessels. In *X. pennsylvanicum* and *X. globosum* the water-conducting tissue composes 2 per cent of the stem area, and in *X. americanum* 3 per cent.

Wood parenchyma is scanty in amount (figs. 38, 39, 40), and the stem is made strong by a large amount of lignified wood fibers (fig, 42, *P*). In *X. pennsylvanicum* 90.5 per cent of the xylem is lignified, in *X. americanum* 85.1 per cent, and in *X. globosum* 86.7 per cent.

The wood fibers average in diameter .0113 mm. in *X. pennsylvanicum*, and .0110 mm. in *X. americanum* and in *X. globosum*.

The wood parenchyma also contains glucosides and calcium oxalate crystals.

The cambium cells are not always discernible, and do not form a complete ring.

The phloëm is made up of a mass of very irregular thin-walled cells (fig. 34), which are not differentiated into sieve tubes and companion cells. These thin-walled cells vary in diameter from .004 mm. to .020 mm. in *X. pennsylvanicum,* from .005 mm. to .022 mm. in *X. americanum,* and from .005 to .020 mm. in *X. globosum.*

The phloëm is rich in cell centents, containing glucosides, a rather small amount of protein, and numerous bodies which give the mucilage stain with methylene blue.

In each species a starch sheath is discernible (figs. 35, 36, 37), the cells containing very large crystals of calcium oxalate (fig. 35, *P*) and rather large starch grains (fig. 36, *T*), which are more numerous in *X. americanum* than in the other species. In transverse section the cells of the starch sheath have an average diameter of .0357 mm. in *X. pennsylvanicum,* .0270 mm. in *X. americanum,* and .0261 mm. in *X. globosum.*

The medullary rays vary in width from 3 to 9 cells in *X. pennsylvanicum,* from 2 to 6 in *X. americanum,* and from 2 to 5 in *X. globosum,* with an average width in number of cells of 4.5 for *X. pennsylvanicum,* 2.5 for *X. americanum,* and 3.4 for *X. globosum.* The ray cells of the xylem are lignified and have pitted walls. They have an average radial diameter of .0254 mm. in *X. pennsylvanicum,* of .024 mm. in *X. americanum,* and of .027 mm. in *X. globosum.*

There is a striking variation in the size and shape of the different bast groups seen in one cross section of a single species (fig. 26).

In one cross section the bast masses varied in size from a mass .045 mm. in diameter composed of only six cells, to a mass .570 mm. in diameter made up of many cells. All bast cells are lignified with strongly pitted walls .004 to .005 mm. in thickness.

The parenchyma of the pericycle is composed of large thin-walled cells and contains numerous air spaces (fig. 49). Here frequent resin ducts occur (fig. 49, *T*) surrounded by, on the average, six very small secreting cells (fig. 50, *X*), which are rich in cell contents. They contain a very small amount of protein, small masses which stain a deep blue in methylene

blue, and when sections were treated with Sudan III bodies were found in these cells which seemed to be oil.

The parenchyma of the pericycle contains glucosides, calcium oxalate crystals (fig. 53), and numerous oval bodies (fig. 52) which stain for mucilage with methylene blue, and give the characteristic red color with alcannin and Sudan III.

The collenchyma, in cross section from 4 to 6 cells deep (figs 49, *R;* 50, *V*), is composed of comparatively small thick-walled cells with minute air spaces at the corners, which are rather more numerous in *X. pennsylvanicum* than in *X. americanum* or *X. globosum.* In each species the subepidermal layer (figs. 49 and 50) resembles the epidermis and is richer in cell contents than other cells of the collenchyma.

In cross section (figs. 57 and 58) the diameter of the epidermal cells parallel with the circumference of the stem is on the average .0197 mm. in *X. pennsylvanicum,* .0140 mm. in *X. americanum,* and .0190 mm. in *X. globosum.* In longitudinal section the diameter averages .0428 mm. in *X. pennsylvanicum,* .0516 mm. in *X. americanum,* and .0460 mm. in *X. globosum.* The epidermal cells of each species contain numerous glucoside bodies. In tangential section they appear either in the form of single droplets or masses (figs. 55, *D;* 56, *F*), or in the form of clusters of minute droplets.

Slight cutinization occurs in the outer walls of the epidermal cells. *X. pennsylvanicum* and *X. globosum* have a ridged cuticle .0015 mm. in thickness, while the cuticle in *X. americanum* is of the same thickness but less ridged.

Two forms of hairs similar to those found on the leaf occur on the stem (fig. 54). In *X. pennslyvanicum* both forms are very scanty, while in *X. americanum* there are on the average 2.57 large and 4 small linear hairs to the sq. mm., and in *X. globosum* 1.17 large and 1 small linear hair to the sq. mm.

ROOT.

The cross section of the tap root shows a protostele figs. 60, 61) with vessels varying in diameter from .025 to .135 mm. in *X. pennsylvanicum,* from .025 to .150 mm. in *X. americanum,* and from .015 to .120 in *X. globosum.* In cross section the ray cells are generally composed of three rows of cells, the central row made up of ray tracheids with bordered pits. All cells of the xylem have lignified walls.

The phloëm varies in thickness in the three species. In *X. pennsylvanicum* it is .150 mm., in *X. americanum* .202 mm., and in *X. globosum* .195 mm. in thickness.

In *X. pennsylvanicum* and *X. globosum* the phloëm at two opposite sides of the root is thicker than at other parts of the root (figs. 60 and 61), and extends into the xylem. *X. americanum* does not have this characteristic. The phloëm in each species has irregular areas in which the cell walls are lignified.

SUMMARY.

1. The three types of *Xanthium* described present some striking external characteristics, while in their anatomy there are some definite but minor differences which might prove of uncertain value in classification.

2. In each species three large veins enter the leaf, the midrib and other large veins containing a very large amount of thin-walled parenchyma cells in which resin ducts occur.

3. The leaf of *Xanthium pennsylvanicum* has a little more palisade tissue than the leaf of *Xanthium americanum* and *Xanthium globosum*.

4. The stomata are more numerous in *X. americanum* than in *X. pennsylvanicum* or *X. globosum*.

5. Numerous trichomes of two different forms occur on both surfaces of the leaf. The most conspicuous is relatively large, awl-shaped, and composed of three cells; the other form is linear. These always occur in connection with the water-storage cells above and below the veins.

6. A cuticle layer covers the entire outer wall of the hairs in each species.

7. The young stems are slightly five-angled in outline, with *X. globosum* containing a little more wood than the other two species described.

8. *X. americanum* and *X. globosum* have the ray cells between the bases of the bundles elongated tangentially. *X. pennsylvanicum* does not show this characteristic.

9. *X. americanum* has 3 per cent of the stem area given to vessels; *X. pennsylvanicum* and *X. globosum* have 2 per cent.

10. The phloëm is not differentiated into sieve tubes and companion cells.

11. In each species resin ducts occur in the parenchyma of the pericycle.

12. The starch sheath is discernible in each species, and contains starch grains and calcium oxalate crystals.

13. The epidermis of each species contains glucosides in the form of single droplets or of clusters of droplets.

14. Each species has a ridged cuticle.

15. Hairs similar to those found on the leaf occur on the stem, numerous in *X. globosum* and *X. americanum,* but scanty in *X. pennsylvanicum.*

16. Glucosides occur in each species in the phloëm, the parenchyma, the wood parenchyma and the pith.